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Development of a Selective Broth Medium for the Detection of Injured *Campylobacter jejuni* by Capacitance Monitoring

J. ERIC LINE* AND KIRSTEN G. PEARSON

U.S. Department of Agriculture, Russell Research Center, P.O. Box 5677, Athens, Georgia 30677, USA

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ABSTRACT

The purpose of these studies was to develop a conductimetric method for the rapid detection of *Campylobacter jejuni.* Numerous basal medium components were analyzed to develop a growth-enhancing broth medium for detection of freeze-injured *Campylobacter* cells using a conductimetric system. The final medium was composed of a modified Campy-Line agar from which the agar and triphenyltetrazolium chloride were removed and the amino acid, L-arginine was added. Pure isolates of *C. jejuni.* (frozen and thawed to produce stressed cells) were utilized to test the detection methodology. Monitoring of significant changes in the capacitance signal was found suitable for detection of *Campylobacter* proliferation. Using stressed pure cultures, *Campylobacter* growth was repeatedly detected at very low inoculum levels (about one cell per well). There was a direct linear relationship between detection times (DTs) and the initial inoculum level. For example, using a single strain, the mean DT ($n = 20$) at the 10 CFU/ml inoculum level was 28.6 h, with 100% of the inoculated wells detecting. The mean DTs at the 100, 1,000, and 10,000 CFU/ml inoculum levels were 24.9, 21.4, and 17.0 h, respectively. This study demonstrates that conductimetric methods can be utilized for the rapid detection of *C. jejuni.*

Campylobacters are foodborne pathogens of primary importance. The Centers for Disease Control and Prevention indicated that, in 1999, the incidence of human campylobacteriosis was 17.5 cases per 100,000 persons *(2).* To improve food safety, the need exists for rapid, automated methods of detecting viable campylobacters in foods. It is especially difficult to detect stressed cells that can result from processing plant conditions *(7).* Traditional enumeration of campylobacters involves time-consuming most-probable-number procedures, requiring at least 72 h for analysis, or direct plating methodologies, requiring about 48 h to obtain results *(8).* Both of these methods require incubation of the sample under microaerobic conditions (10% CO_2, 5% O_2, 85% N_2). The purpose of this study was to develop a simple, rapid method and broth medium for *Campylobacter* detection using conductimetric methods without providing modified atmospheric conditions for growth.

Microbiological conductimetric methods are used often in the food and cosmetic industries to test shelf life and monitor bacterial growth; these methods provide results in a timely and efficient manner. Conductimetric instruments monitor microbial metabolism inside a growth medium by the measurement of significant changes in electrical activity (impedance, conductance, or capacitance *(5)*). Impedance and conductance both involve the passage of charged ions between two electrodes, whereas capacitance measures the storage of an electrical charge at the electrode-medium interface. Many factors can affect the electrical nature of a medium when monitoring microbial growth, including cell density, temperature, and conductivity of the medium *(6).* To successfully develop a medium for detection of a specific organism, all factors influencing the electrical charge, as well as the growth requirements of the organism being tested, must be taken into account. Cappelier et al. *(1)* demonstrated the application of conductimetric technology to detect growth of campylobacters by monitoring changes in conductance of the medium. Several studies have shown that capacitance signals provide a better indicator of microbial growth, even though changes in capacitance at the electrode-medium interface are not clearly understood *(5).* We used an instrument capable of measuring any of the three electrical signals so that we might compare and optimize methods for detection of campylobacters. Preliminary studies in our laboratory demonstrated that monitoring changes in the capacitance signal provided a more sensitive indicator of campylobacter growth than either conductance or impedance signals. It was hypothesized that the medium volume in the instrument wells could be critical to *Campylobacter* detection because growth near the electrodes could give the best signal. The method was then developed in three phases: determination of the optimum broth medium and overlay volumes to produce the best signal, optimization of the broth medium itself, and determination of the relationship between inoculum level and time to detection.

MATERIALS AND METHODS

We used a bactometer microbial monitoring system (bioMerieux, Hazelwood, Mo.) to monitor conductimetric changes indicating microbial growth. Each bactometer module contains 16 wells of about 2 ml total volume each, with two electrodes exposed in the well. One incubation chamber holds eight modules.

Determination of optimum broth and overlay volumes. The necessity of providing an external microaerobic atmosphere

* Author for correspondence. Tel: 706-546-3522; Fax: 706-546-3771; E-mail: eline@saa.ars.usda.gov.

TABLE 1. *Final formulation of broth medium used in conductimetric experiments*

Ingredient	Quantity (per liter)
Brucella broth	28.0 g
Ferrous sulfate	0.5 g
Sodium bisulfate	0.2 g
Pyruvic acid	0.5 g
Alpha-ketoglutaric acid	1.0 g
Sodium carbonate	0.6 g
Yeast extract	3.0 g
L-Arginine	0.1 g
Supplement[a]	
Hemin	2 ml
Cefoperazone	200 mg
Nystatin	50 mg
Rifampicin	10 mg
Vancomycin	10 mg
Trimethoprim	5 mg
Polymyxin	0.35 mg

[a] Preparation of supplements was as described by Line *(4)* for making Campy-Line agar (CLA).

was overcome by incorporating into the media ferrous sulfate, sodium metabisulfite, and sodium pyruvate (FBP), as described by George et al. *(3),* for increasing the oxygen tolerance of *Campylobacter jejuni* and then sealing off the well surface using a sterile mineral oil overlay of at least 0.5 ml. To determine the optimum broth and overlay volumes for detection of campylobacters, two simple experiments were conducted. Inocula were prepared from frozen campylobacter stock cultures (*C. jejuni* ATCC 49943 [Manassas, Va.] and a wild-type *C. jejuni* isolated in this laboratory from a chicken carcass rinse [PMS 07]). *Campylobacter* suspensions were prepared by harvesting pure isolates from 42-h Brucella-FBP agar plates (Oxoid, Ogdensburg, N.Y.). A spectrophotometer was used to prepare solutions at a standard optical density of about 10^8 CFU/ml for each strain. Serial dilutions were then prepared in sterile Campy-Line broth (CLB) prepared as Campy-Line agar *(4)* excluding the agar and triphenyltetrazolium chloride to produce final concentrations of 10^2 and 10^4 *Campylobacter* cells per ml. Quadruplicate wells were filled with 0.8, 1.0, 1.2, or 1.4 ml of inoculated CLB containing 10^2 or 10^4 cells/ml of either strain. Negative control wells were filled with representative volumes of sterile CLB. All wells were sealed with sterile mineral oil overlays (0.5 ml) and capped with plastic lids. Filled modules were immediately placed in the bactometer incubation chamber at 42°C; changes in capacitance were monitored for 72 h. A similar second experiment was conducted where the medium volume in the wells was held constant at 1.4 ml and the oil overlay volume was varied from 0.2 to 0.8 ml.

Broth medium optimization. Component ingredients of standard Campy-Line agar (without agar and triphenyltetrazolium chloride) were adjusted and amended in a series of trials to optimize a broth medium for conductimetric assays. A variety of compounds were tested at several concentrations to determine their influence on growth of freeze-injured campylobacter cells, as indicated by capacitance monitoring. Those compounds tested included: tryptone, soytone, lactalbumin hydrolysate, yeast extract, sodium chloride, sodium bisulfite, sodium carbonate, ferrous sulfate, pyruvic acid, alpha-ketoglutaric acid, succinic acid, L-arginine, L-aspartic acid, L-cysteine, L-serine, L-threonine, formic acid, fumaric acid, maleic acid, oxaloacetic acid, mucin, NAD and hemin (all from Sigma Chemical Co., St. Louis, Mo.). Media were prepared in 250-ml volumes on the day of use. Basal ingredients were autoclaved at 121°C for 15 min at 1.47 kg/cm^2 (21 psi), and then cooled to approximately 40°C in a water bath prior to addition of supplements (hemin, antibiotics) and adjustment to pH 7.4 (±0.2) using sterile 10 N NaOH. Each module well was aseptically filled with 1.4 ml of the appropriate medium being analyzed.

TABLE 2. *Influence of Campy-Line broth (CLB) volume and oil overlay volume (CLB/oil overlay, ml/ml) on mean (*n = *4)* Campylobacter *detection times (h)*

C. jejuni strain	Inoculum level (CFU/ml)	Detection time (h)			
		0.8/0.5	1.0/0.5	1.2/0.5	1.4/0.5
ATCC 49943	10^2	18.6	17.4	16.8	13.8
PMS 07	10^2	20.1	19.0	17.1	17.1
ATCC 49943	10^4	12.1	10.9	8.5	8.5
PMS 07	10^4	11.7	11.9	10.8	9.3

Two pure *C. jejuni* isolates were injured by a single freeze (−70°C) and thaw (25°C) cycle and were used to test the recovery and growth efficiency of the various media developed. *C. jejuni* ATCC 49943 was again used, as well as an isolate (87-02-096) provided by our colleagues in France who had conducted some preliminary testing (BioMerieux, Lyon, France). Frozen cell suspensions were thawed at room temperature on the day of use. Stock *Campylobacter* populations were predicted to be at least 10^9 CFU/ml when placed into the freezer, with an approximate 3-log loss after freeze-thaw, resulting in an initial starting population of about 10^6 CFU/ml. Brucella agar plates (Oxoid, Ogdensburg, N.Y.) were inoculated with serial dilutions from each cell suspension and incubated (42°C, microaerobically) for 48 h to confirm the populations.

Quadruplicate wells containing 1.4 ml of the media to be tested were individually inoculated with 100 μl of either cell sus-

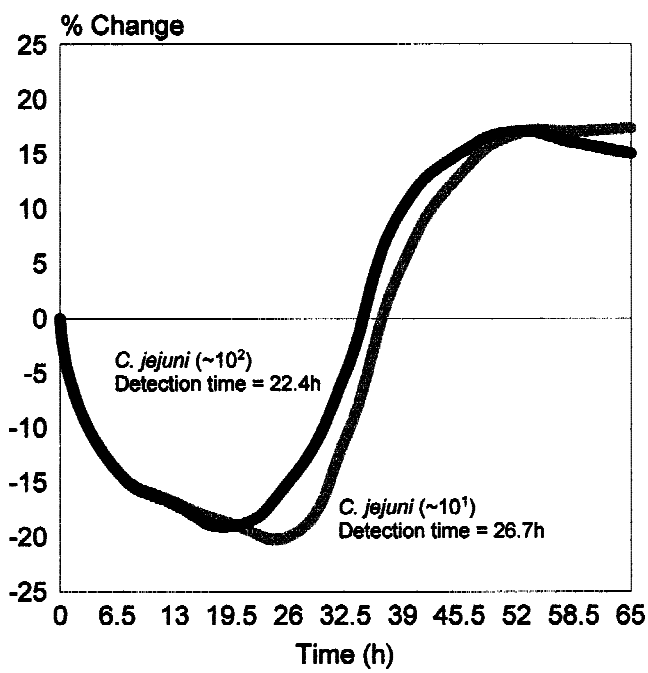

FIGURE 1. *Typical capacitance curves for* C. jejuni *inoculated into bactometer wells at 10^1 or 10^2 CFU/ml.*

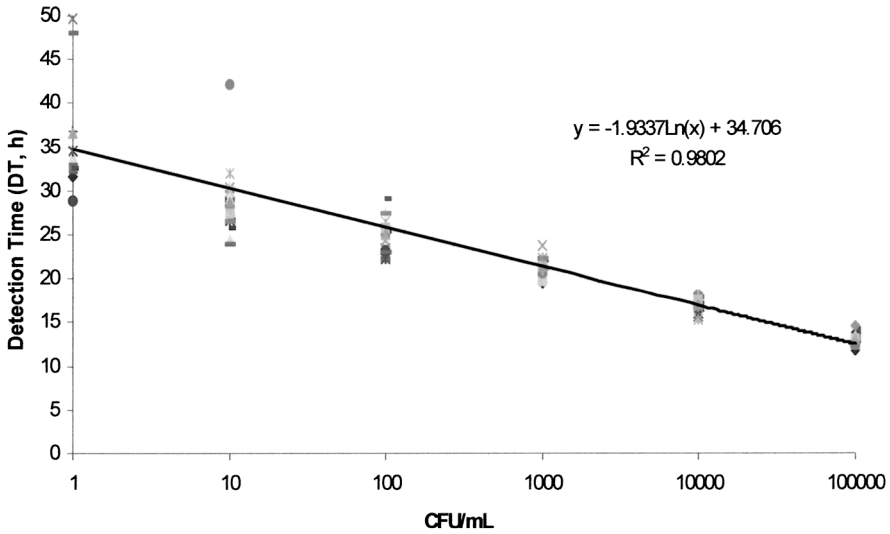

FIGURE 2. *Influence of inoculum level on detection time for* C. jejuni *(87-02-096).*

pension and then sealed using a sterile mineral oil overlay (0.5 ml). One well from each module containing only a sterile medium and mineral oil overlay served as a negative control. Modules were then placed inside the incubation chamber at 42°C for at least 48 h. Detection times (DTs) were recorded by the instrument using capacitance mode. Mean DTs were calculated for each strain and medium type.

Determination of relationship between DT and inoculum level. To determine the relationship between DT and the number of *Campylobacter* inoculated into the wells, a study was conducted using similar methods as before. *C. jejuni* cultures (ATCC 49943 or France isolate 87-02-096) were serially diluted and used to inoculate approximately 1, 10, 100, 1,000, 10,000, or 100,000 *Campylobacter* cells into 20 replicate wells containing 1.4 ml of the optimum enrichment broth developed: modified CLB with arginine (Table 1). The wells were sealed with 0.5-ml sterile mineral oil, and changes in the capacitance signal were monitored for 72 h at 42°C.

RESULTS AND DISCUSSION

Initial studies conducted to determine optimum broth volumes revealed that DTs decrease with increasing broth volumes up to 1.4 ml with the use of a constant 0.5-ml oil overlay (Table 2). It is possible that the increased broth volume in the wells brought the area of the medium where the *Campylobacter* was growing closer to the electrodes, hence decreasing DTs. Mean DTs as low as 13.8 h were recorded for initial inocula of 10^2 *Campylobacter* and as low as 8.5 h for 10^4 *Campylobacter.* Variations in DTs between replicate wells were usually small, and no false positives were recorded in the negative control wells. Some differences in DTs were noted between the two *Campylobacter* strains tested. The oil overlay method provided enough of a microaerobic environment to allow *Campylobacter* growth without the necessity of providing an artificial atmosphere in the incubation chamber. Results from a similar study using a constant volume of CLB (1.4 ml) and varying oil overlay volumes (from 0.2 to 0.8 ml) demonstrated that overlay volumes between 0.4 and 0.6 ml give the shortest DTs.

Several modifications of the standard CLB medium formulation (without agar and triphenyltetrazolium chloride) were evaluated for an ability to enhance *Campylobacter* detection times using the bactometer system. Many of the medium formulations tested made it possible to clear-

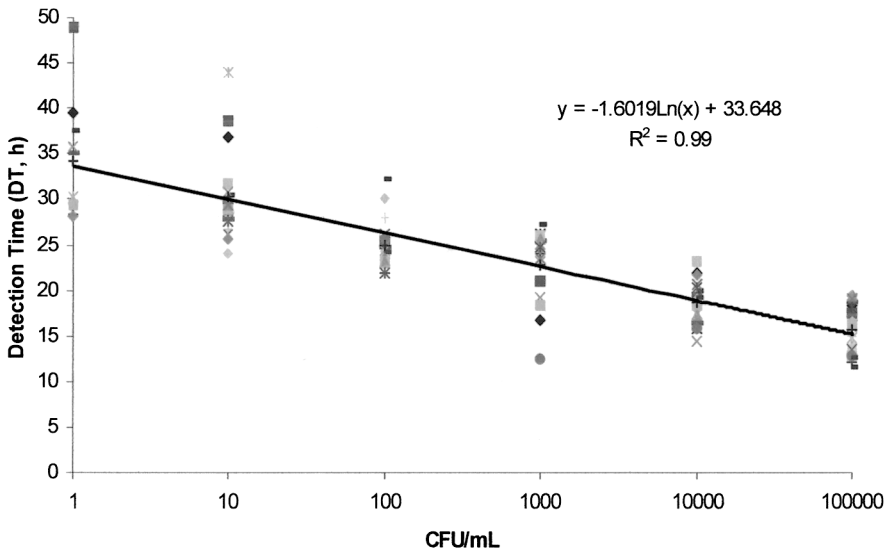

FIGURE 3. *Influence of inoculum level on detection time for* C. jejuni *(ATCC 49943).*

ly monitor changes in the capacitance signal (Fig. 1). Amendment of the media with several amino acids, including L-arginine, L-serine, L-cysteine, and L-threonine, contributed to enhanced detection times and resuscitation of injured cells, with arginine offering the most benefit. For this reason, CLB without agar and triphenyltetrazolium chloride and containing 100 mg arginine per liter was used for the remaining studies (modified CLB, Table 1).

An inverse relationship was determined between *Campylobacter* inoculum levels in the wells and DTs. For example, when using the freeze-stressed *C. jejuni* (France isolate 87-02-096), the mean DT ($n = 20$) at the 10 CFU/ml inoculum level was 28.6 h, with 100% of the inoculated wells detecting. The mean DTs at the 100, 1,000, 10,000, and 100,000 CFU/well inoculum levels were 24.9, 21.4, 17.0, and 13 h, respectively (Fig. 2). Such results indicate that a standard curve could be plotted enabling the use of DTs to more rapidly predict *Campylobacter* populations in a sample than traditional methods. The method was also very sensitive. Using stressed, pure cultures, *Campylobacter* growth was repeatedly detected at very low inoculum levels (about one cell per well). Similar results were obtained for the other *C. jejuni* strain (ATCC 49943) tested (Fig. 3). This work was conducted with pure cultures of *Campylobacter*. Further studies are needed to adapt this method to the rapid detection of *Campylobacter* spp. in poultry and other food products, where more challenging competing microflora can be present.

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